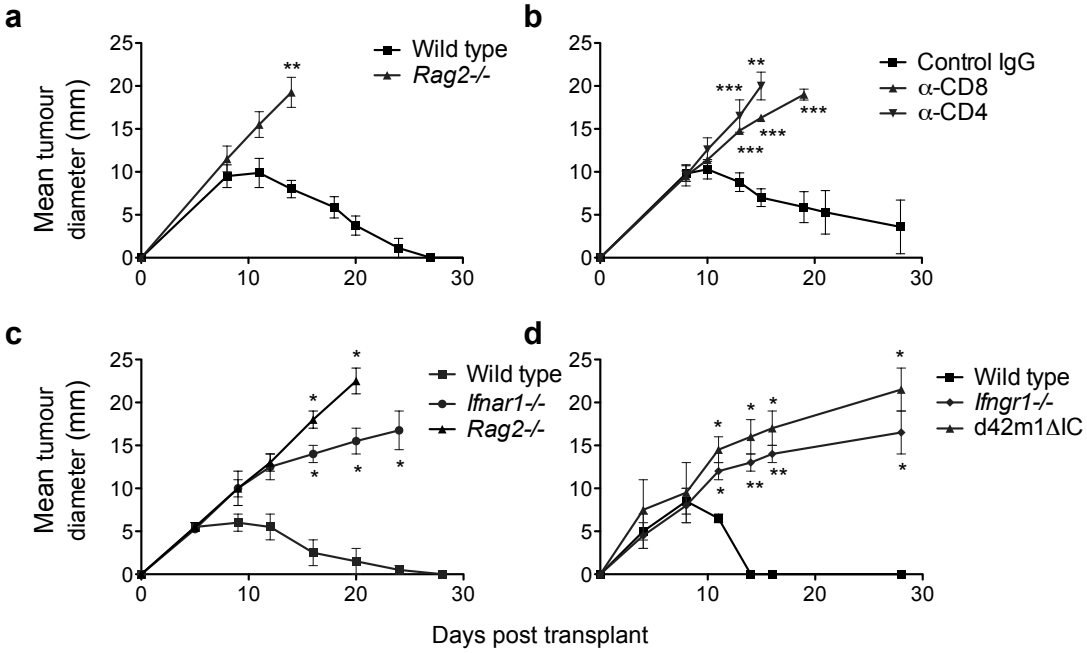
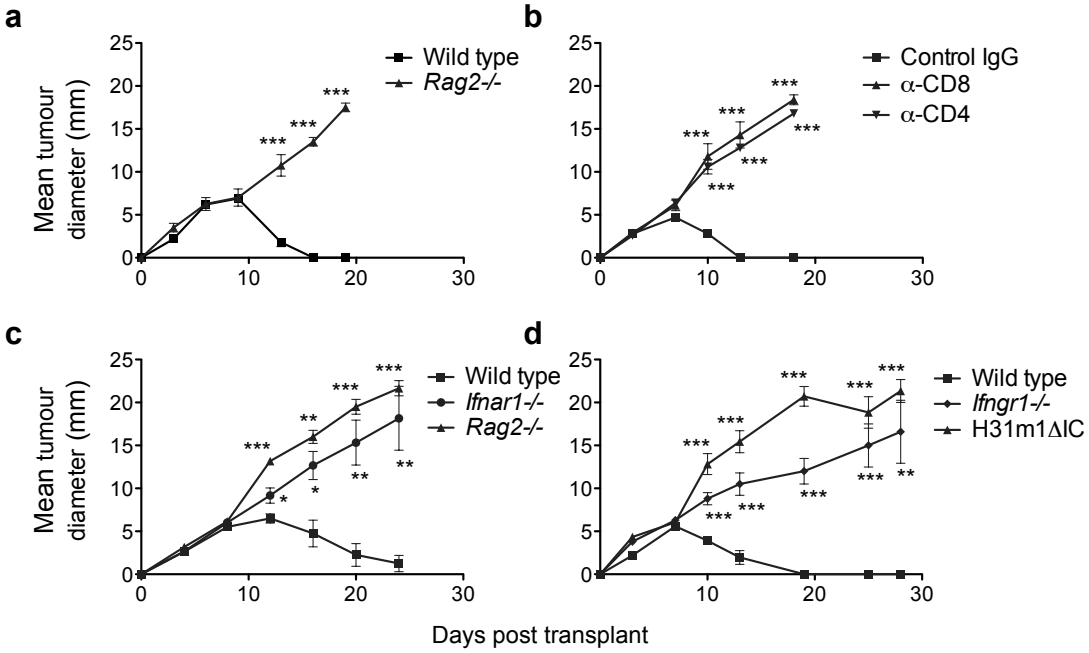
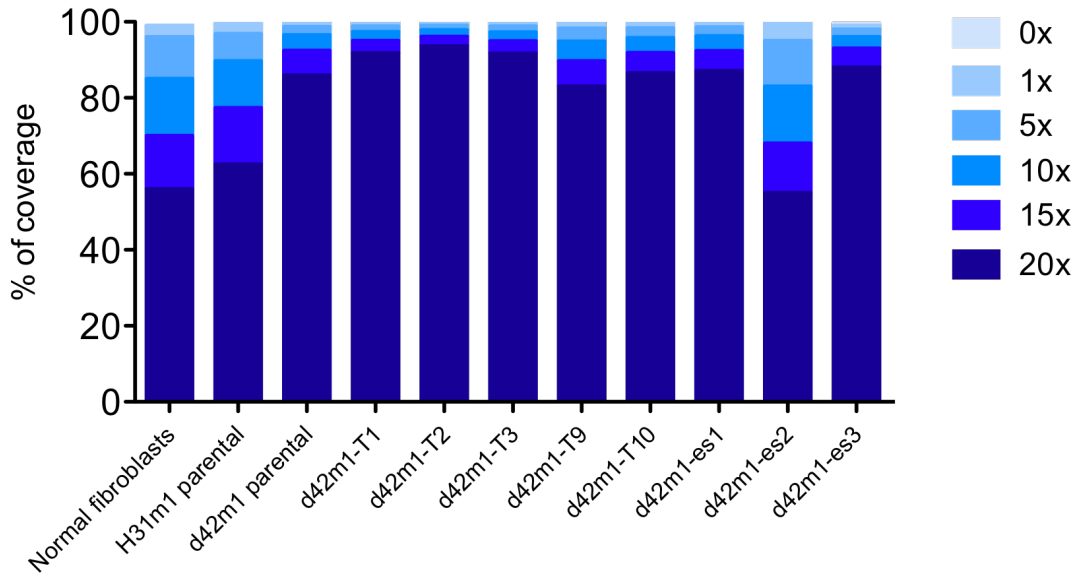


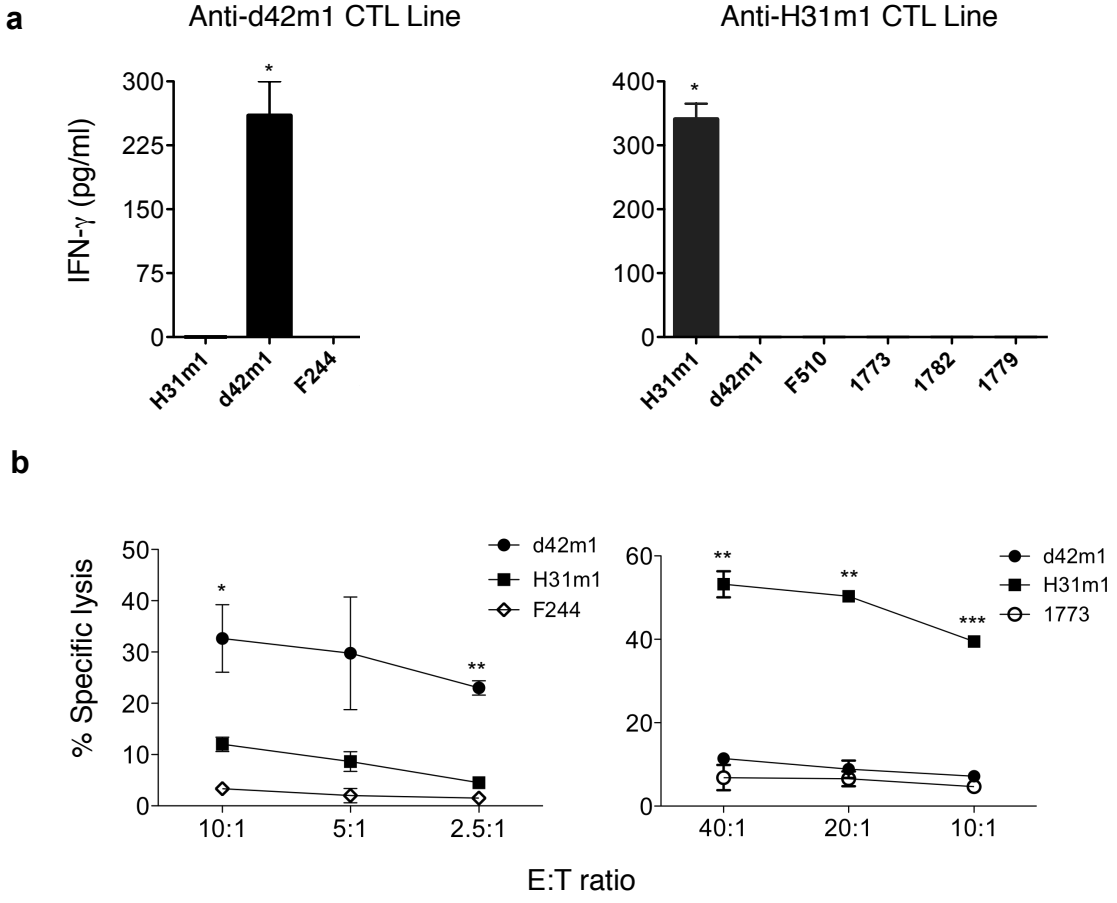
Supplementary Figure 1. d42m1 is a highly immunogenic unedited sarcoma. d42m1 tumour cells were injected subcutaneously at a dose of $1x10^6$ cells into syngeneic wild type (**a-d**), *Rag2*$^{-/-}$ (**a** and **c**), *Ifnar1*$^{-/-}$ (**c**), or *Ifngr1*$^{-/-}$ (**d**) mice and tumour growth was followed over time. **b**, Groups of WT mice injected with $1x10^6$ d42m1 tumour cells were treated with control IgG, anti-CD4, or anti-CD8α mAbs (250 μg) starting at day -1 and then every 7 days thereafter. **d**, d42m1 tumour cells were rendered insensitive to IFN-γ (d42m1ΔIC) by over-expressing a dominant-negative version of IFNGR1 (IFNGR1ΔIC) and were then transplanted ($1x10^6$ cells) into WT mice. Data are presented as average tumour diameter ± s.e.m. of 3-5 mice per group and are representative of at least three independent experiments. Samples were compared using an unpaired, two-tailed Student's *t* test (*p<0.05, **p<0.01, and ***p<0.001).



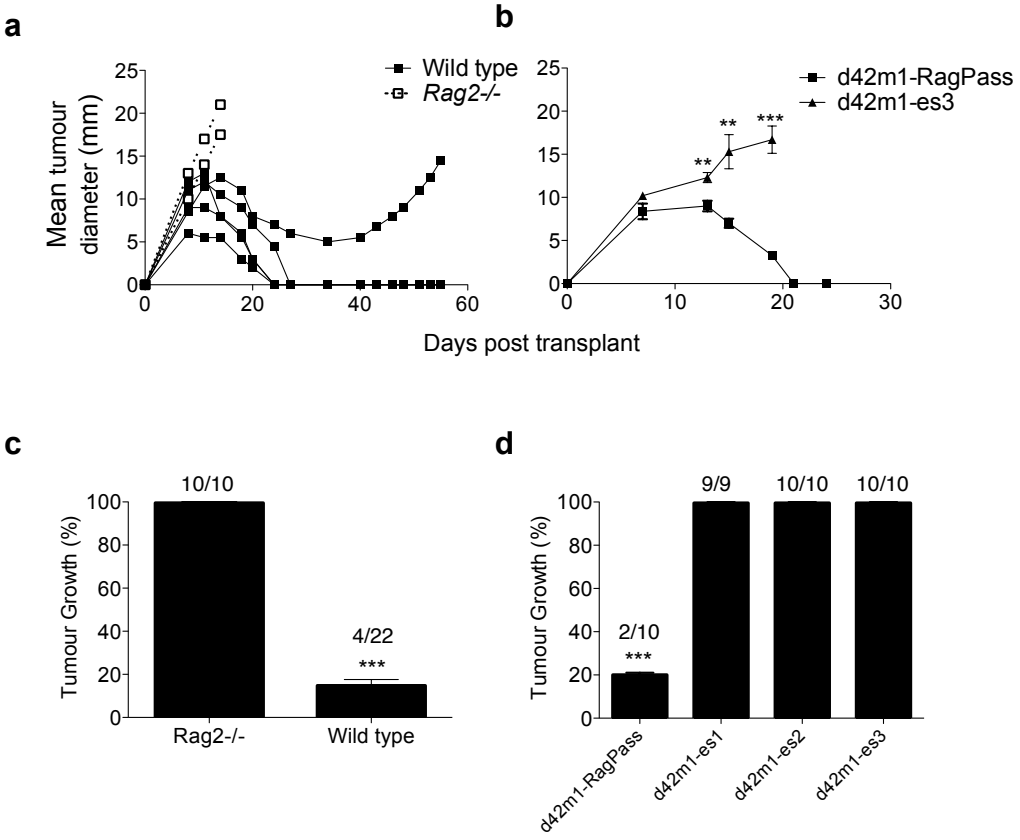
Supplementary Figure 2. H31m1 is a highly immunogenic unedited sarcoma. H31m1 tumour cells were injected subcutaneously at a dose of $1x10^6$ cells into syngeneic wild type (**a-d**), *Rag2*$^{-/-}$ (**a** and **c**), *Ifnar1*$^{-/-}$ (**c**), or *Ifngr1*$^{-/-}$ (**d**) mice and tumour growth was followed over time. **b**, Groups of WT mice injected with $1x10^6$ H31m1 tumour cells were treated with control IgG, anti-CD4, or anti-CD8α mAbs (250 μg) starting at day -1 and then every 7 days thereafter. **d**, H31m1 tumour cells were rendered insensitive to IFN-γ (H31m1ΔIC) by over-expressing a dominant-negative version of IFNGR1 (IFNGR1ΔIC) and were then transplanted ($1x10^6$ cells) into WT mice. Data are presented as average tumour diameter ± s.e.m. of 3-5 mice per group and are representative of at least three independent experiments. Samples were compared using an unpaired, two-tailed Student's *t* test (*p<0.05, **p<0.01, and ***p<0.001).



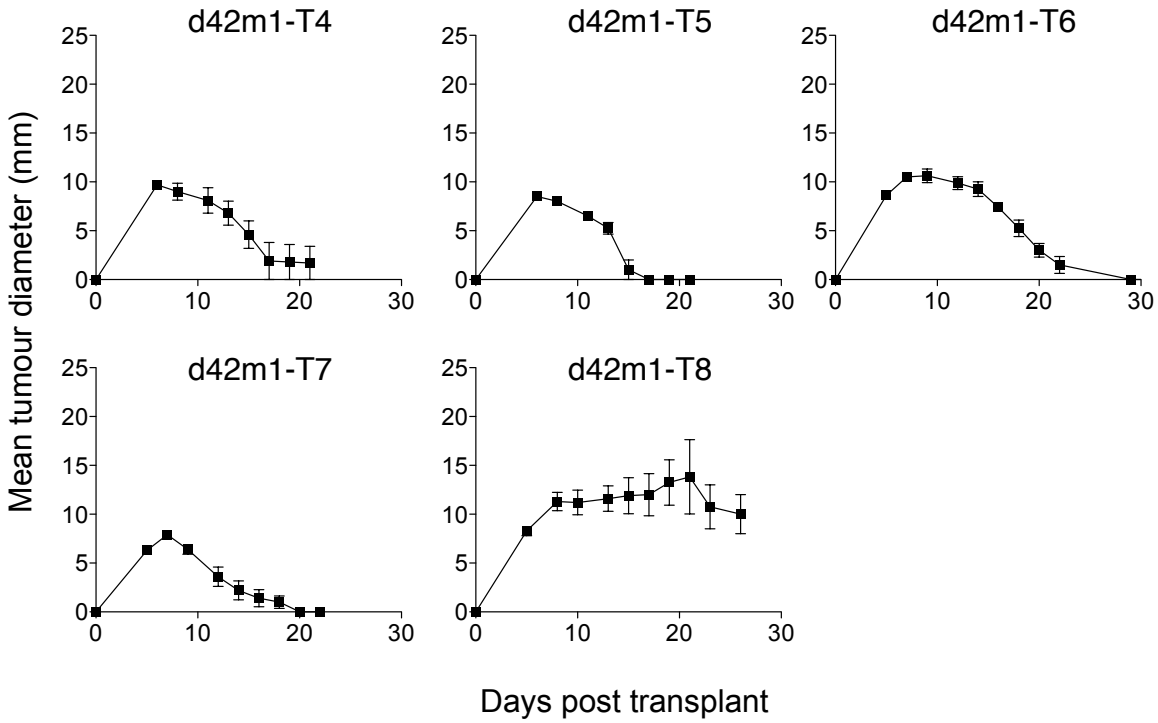
Supplementary Figure 3. Exome sequencing coverage. Percentage of exome sequence coverage (20x, 15x, 10x, 5x, 1x, 0x) is displayed for the MCA sarcoma cell lines and normal skin fibroblasts that were isolated from three independent syngeneic 129/Sv *Rag2*$^{-/-}$ mice.



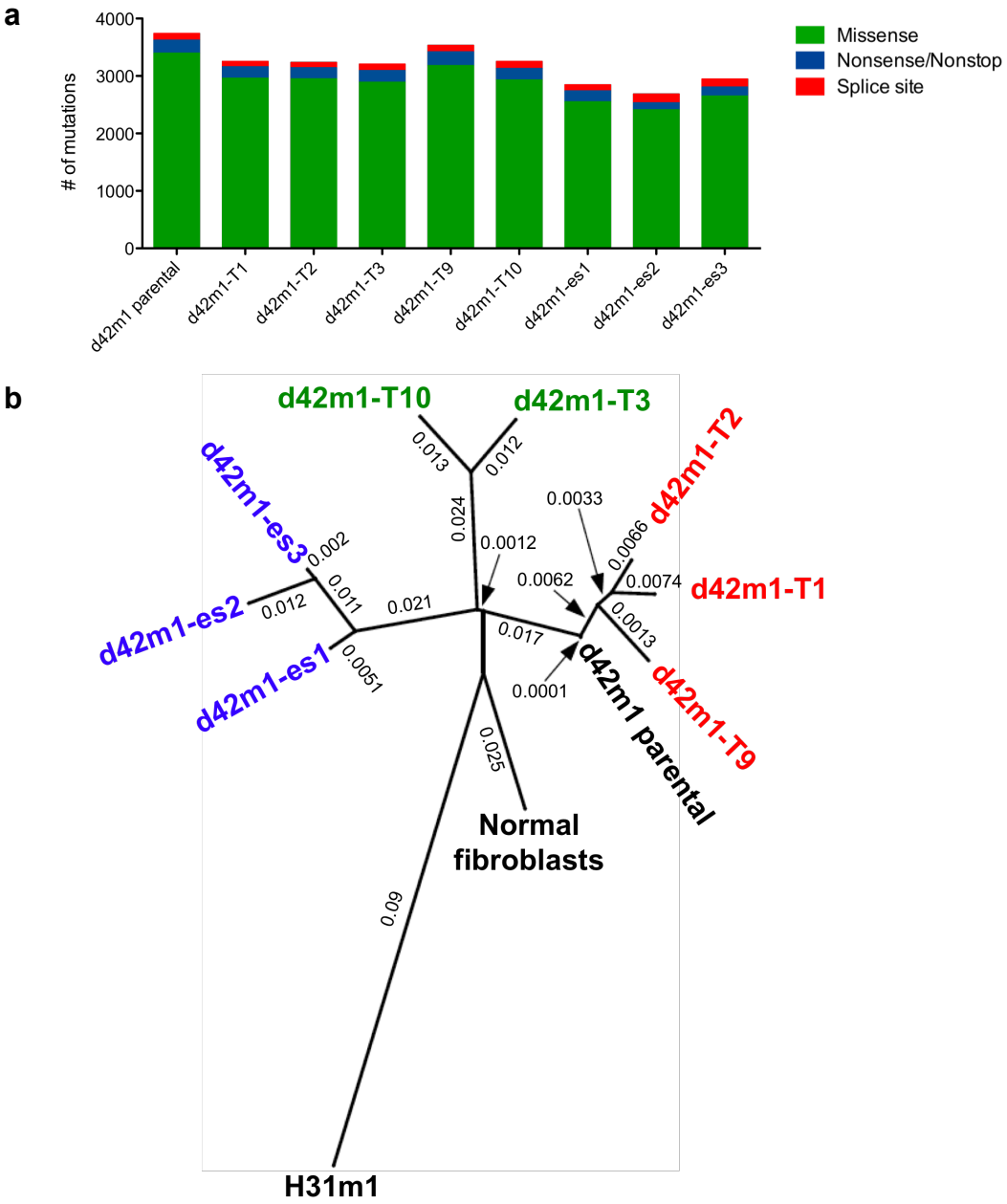
Supplementary Figure 4. H31m1 and d42m1 sacromas are immunogenically distinct. IFN-γ ELISA assay (**a**) and killing assay (**b**) using the bulk CTL lines developed against either parental d42m1 (left panel) or H31m1 (right panel) and tested against unedited MCA sarcoma cell lines, d42m1, H31m1, F510, 1773, 1782, and 1779 or the edited F244 MCA sarcoma derived from a wild type 129/Sv mouse. Data are representative of at least two independent experiments and are presented as average IFN-γ release (**a**) or percent of tumour cell lysis (**b**) ± s.e.m. Samples were compared using an unpaired, two-tailed Student's *t* test (*p<0.05, **p<0.01, and ***p<0.001).



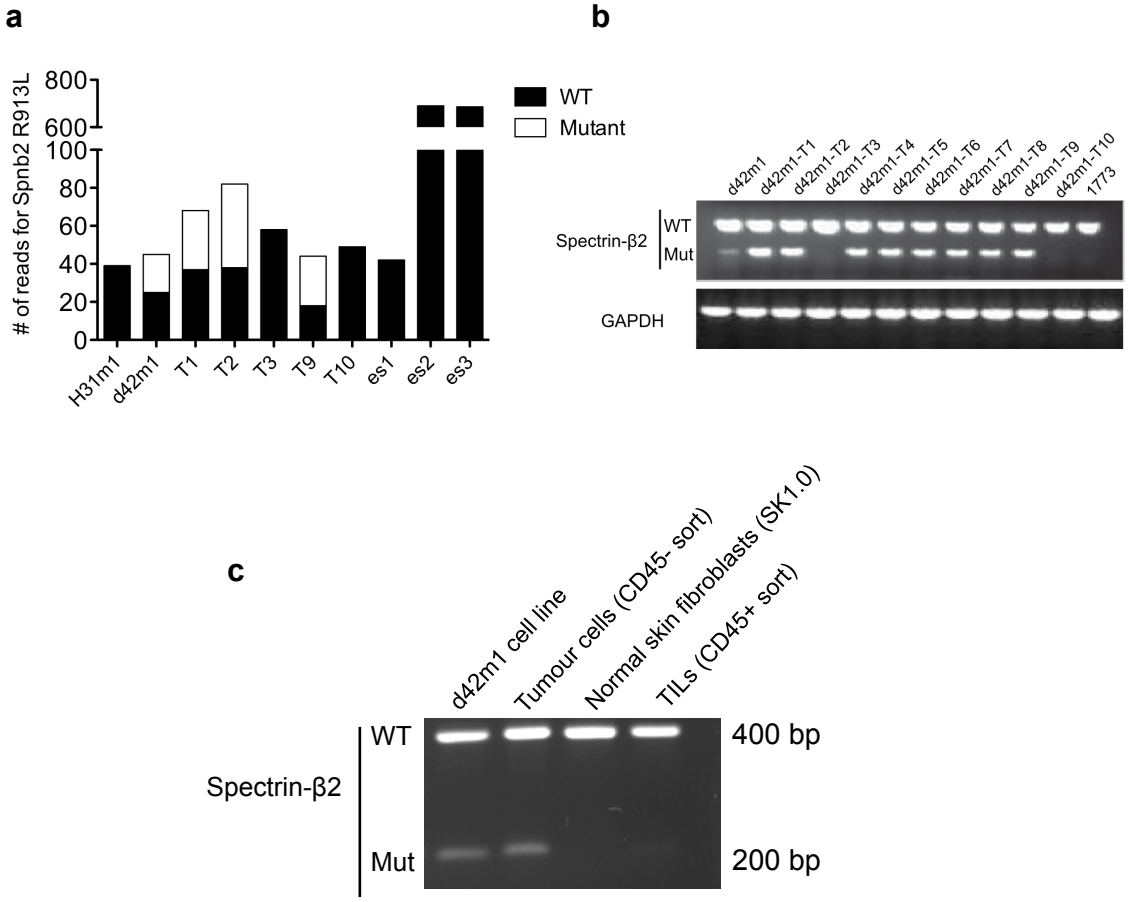
Supplementary Figure 5. Escape tumours of d42m1 display reduced immunogenicity and an edited growth phenotype. a, Growth of d42m1 tumour cells ($1x10^6$) following transplantation into wild type (solid lines) or *Rag2*$^{-/-}$ (dashed lines) mice. **b**, Growth of the escape tumour d42m1-es3 (harvested from the wild type mouse bearing an escaped d42m1 tumour in **a** and generated into a cell line) (n=5, diamonds) or d42m1-RagPass (n=5, squares) following transplantation ($1x10^6$ cells) into WT mice. Data presented as average tumour diameter ± s.e.m of 5 mice per group over time. **c**, Summary of tumour growth in WT mice or *Rag2*$^{-/-}$ mice were challenged with $1x10^6$ d42m1 tumour cells. Data presented as percent tumour positive from 2-4 independent experiments (n=4-6 mice per group). **d**, Summary of tumour growth in WT mice challenged with $1x10^6$ d42m1-RagPass, d42m1-es1, d42m1-es2, or d42m1-es3 tumour cells. Data presented as percent tumour positive from two independent experiments (n=4-5 mice per group). Samples were compared using an unpaired, two-tailed Student's *t* test (**p<0.01 and ***p<0.001).



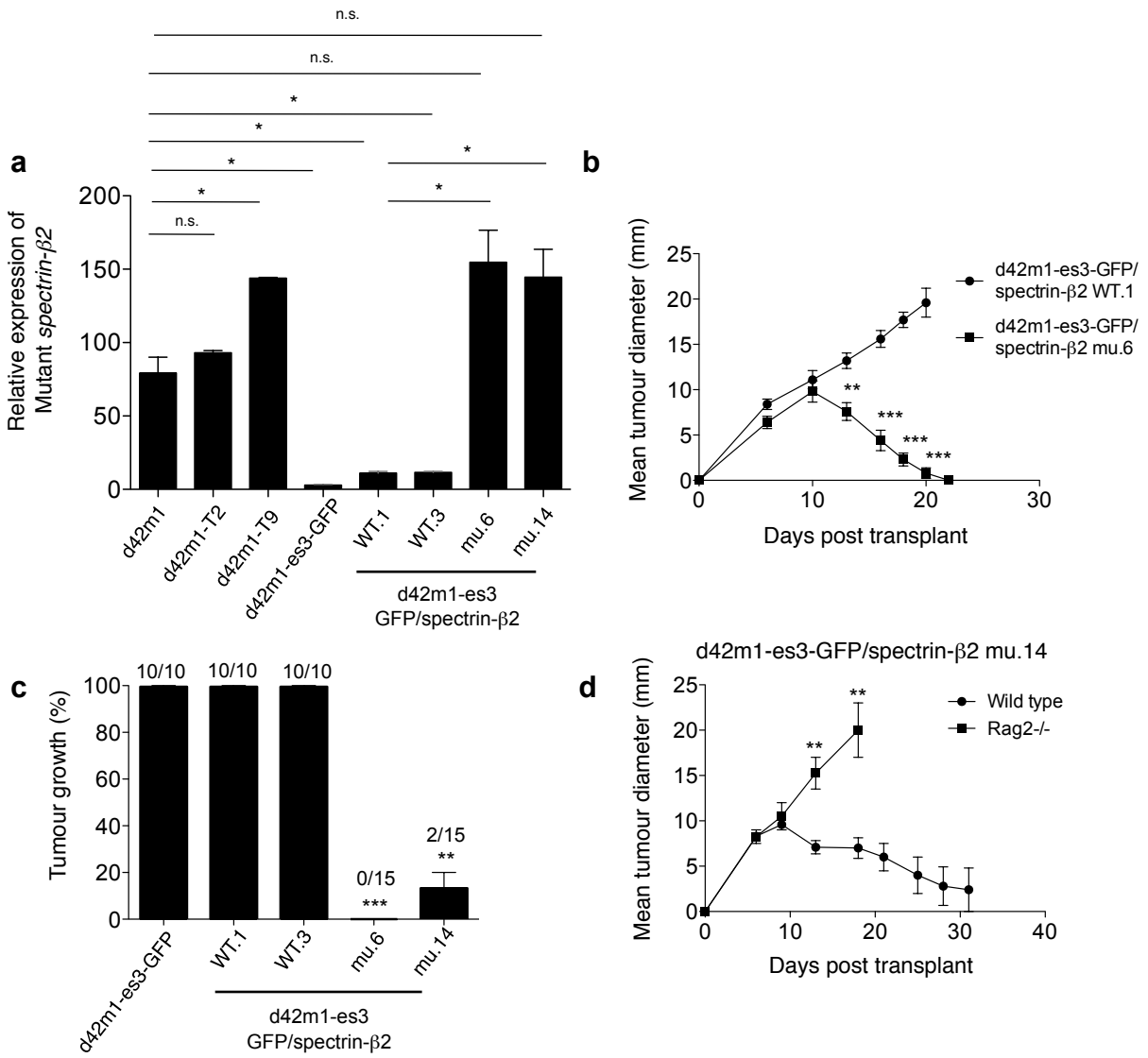
Supplementary Figure 6. Growth phenotypes of additional regressor d42m1 clones. Tumour growth in WT mice challenged with $1x10^6$ d42m1-T4, d42m1-T5, d42m1-T6, d42m1-T7, or d42m1-T8 tumour cells. Data presented as average tumour diameter ± s.e.m of 5 mice per group over time and are representative of at least two independent experiments.



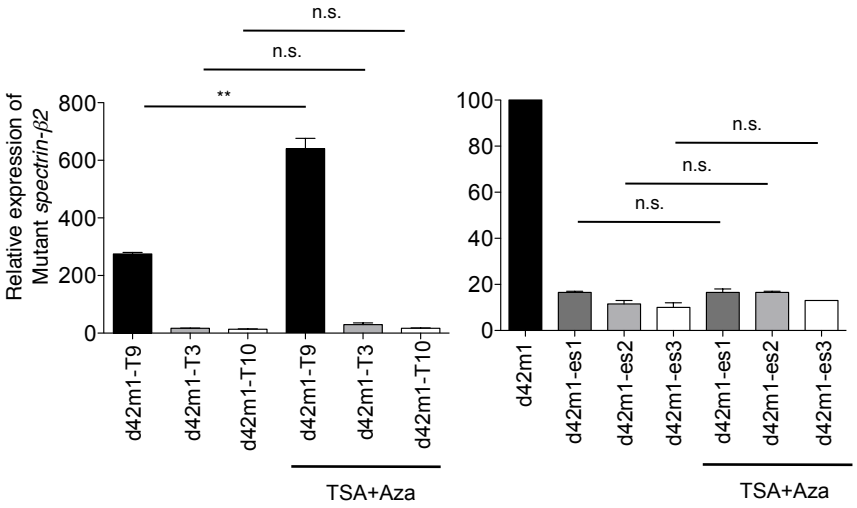
Supplementary Figure 7. d42m1 tumour cell clones and escape tumours are genomically related. **a**, Number and type of somatic, non-synonymous mutations in d42m1 related tumour cells. **b**, Relatedness of normal fibroblasts, H31m1, d42m1 parental cells, d42m1 regressor clones (red), d42m1 progressor clones (green) and d42m1 escape tumours (blue) is displayed in an unrooted phylogenetic tree generated from exome sequencing data using PHYLogeny Inference Package (PHYLIP). Numbers represent the degree of relatedness between samples



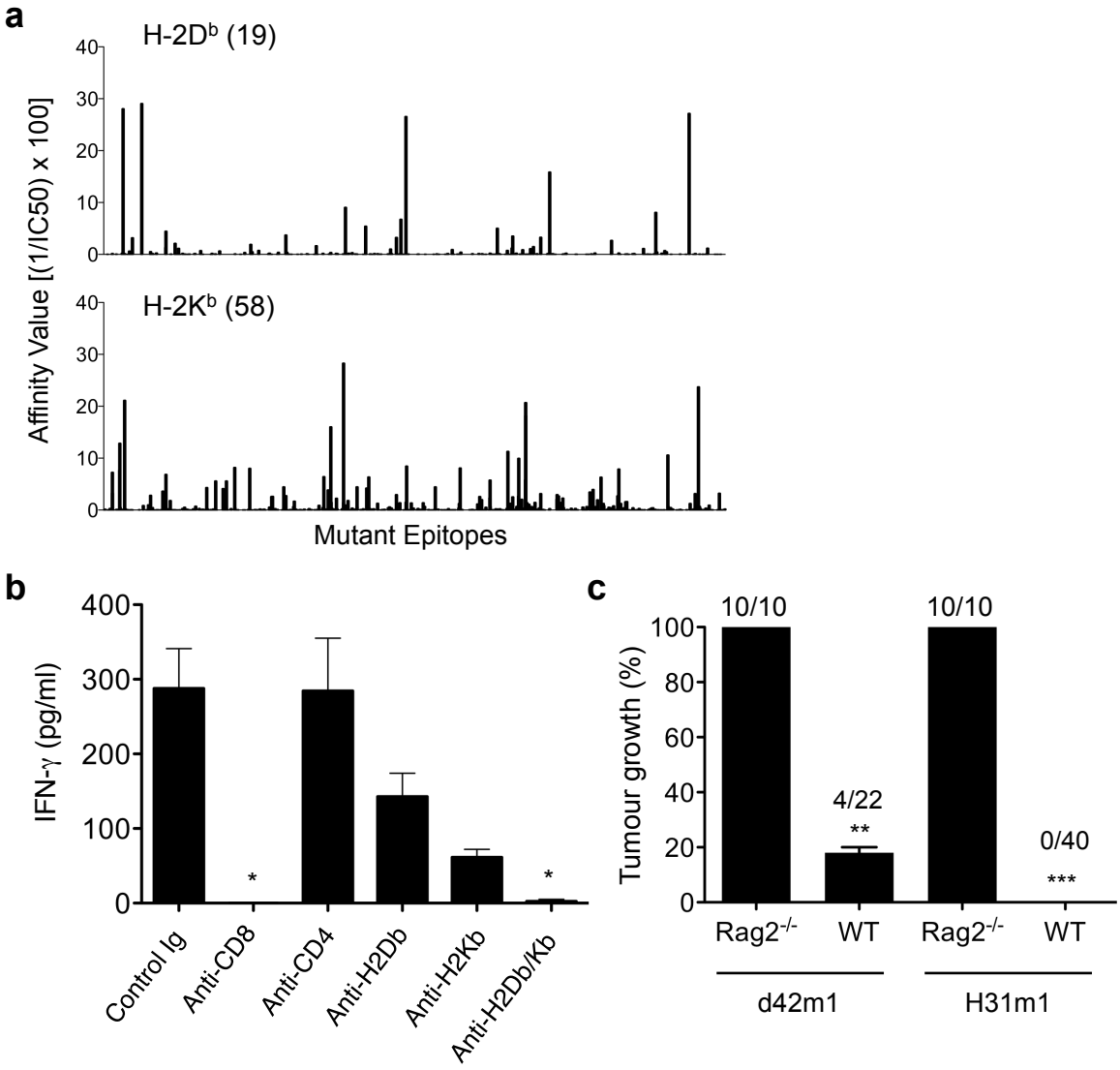
Supplementary Figure 8. The R913L spectrin-β2 mutation in d42m1 tumour cells is a tumour-specific mutation and not a host polymorphism. **a**, Number of WT or mutant spectrin-β2 reads from Illumina cDNA CapSeq. **b,** cDNAs from parental d42m1 or d42m1 tumour cell clones were prepared. After RT-PCR amplification, cDNA fragments were cut by restriction enzyme *Pst1* and analyzed by electrophoresis in 1.2% agarose gels. The G to T point mutation in spectrin-β2 causes a novel restriction site, such that only the R913L mutation will be cleaved by *Pst1* restriction enzymes. **c**, A frozen d42m1 tumour biopsy from the original tumour was thawed and sorted into $CD45^-$ cells (primary d42m1 tumour cells) and $CD45^+$ cells (tumour-infiltrating leukocytes (TILs)). RNA was isolated from sorted cells for cDNA synthesis, which was amplified using primer pairs for spectrin-β2 and digested by *Pst1* as in (**b**). The parental d42m1 cell line and the normal skin fibroblast line SK1.0 which was derived from a syngeneic 129/Sv *Rag2*$^{-/-}$ mouse were also included as positive and negative controls, respectively. Also, these sorted samples were sequenced using 3730 and the results are presented in Supplementary Table 4.



Supplementary Figure 9. Enforced expression of mutant spectrin-β2 in d42m1 escape tumour cells results in rejection in wild type mice. **a**, qRT-PCR analysis using a primer pair specific for mutant spectrin-β2 in d42m1-es3 tumour cell clones that have been engineered to express either wild type or mutant spectrin-β2. Data is displayed as relative expression after normalization to control GAPDH expression and is representative of three independent experiments. **b, d**, Growth of d42m1-es3 tumour cell clones reconstituted with WT or mutant spectrin-β2 and control d42m1-es3 expressing only GFP following transplantation of $1x10^6$ cells into five-member groups of WT (**b, d**) or *Rag2*$^{-/-}$ mice (**d**). Data are presented as average tumour diameter ± s.e.m. over time. **c**, Percent tumour positive mice in five-member groups of WT mice from 2-3 independent experiments is shown. Samples were compared using an unpaired, two-tailed Student's *t* test (*p<0.05, **p<0.01, and ***p<0.001; n.s. is non-significant).



Supplementary Figure 10. Lack of mutant *spectrin-β2* mRNA in d42m1 tumour clones or escape tumours is not due to epigenetic silencing. d42m1 tumour cell clones (left) and d42m1 escape tumours (right) were treated with Trichostatin A (TSA) (0.3 μmol/L) and 5-azacytidine (Aza) (5 μmol/L) for 3 days prior to cDNA preparation. Real-time PCR using a specific primer pair for mutant *spectrin-β2* was performed. Data is displayed as relative expression after normalization of control GAPDH expression and is representative of 3 independent experiments. Samples were compared using an unpaired, two-tailed Student's *t* test (**p<0.01; n.s. is non-significant).



Supplementary Figure 11. H31m1 tumour cells express a number of potential antigens for H-2D^b and H-2K^b. **a**, Missense mutations for H31m1 were analyzed for potential MHC class I neoepitopes that bind to either H-2D^b or H-2K^b. Predicted epitope binding affinities were ultimately expressed as "Affinity Values" (Affinity Value = $1/IC_{50}$ X 100). Number of potential strong affinity binders for each MHC class I molecule are indicated in parentheses. **b**, IFN-γ production by an H31m1-specific CD8$^+$ CTL line following incubation with H31m1 target cells with blocking antibodies against CD8, CD4, H-2D^b and/or H-2K^b. **c**, Percent of tumour growth in *Rag2*$^{-/-}$ or WT mice transplanted with d42m1 or H31m1 tumour cells (1 x 10^6 cells) from 2-5 independent experiments. Samples were compared using an unpaired, two-tailed Student's *t* test (*p<0.05, **p<0.01, and ***p<0.001).